June 26, 1951.

Program: Streak out W-1 on EMB agar. Velvet Print Transfer to plain and to selective medium. When V_1^R clones are detected, take plain incubation for second streaking. Repeat until V_1^R colonies are obtained by indirect selection without exposure to specific selective agent.

P26. Streak out W-1.

A27. Restreak single colonies. Make test ~~transfer~~ Prints

A. Preliminary test (whole culture not recently reisolated: more likely to produce clones from V_1^R in vitro culture). A2:

Transfer somewhat smeared.

Tr. 1. 21 V_1^R } 6 congruences, presumably clones
Tr. 2. 13 V_1^R }

1. Pool pickings from homologous sites on plain transfer plate. Restreak. A28

B } Single colony platings. ~~Transfer~~ Print to P_1, T_1 1, 2
C }
D }

B. 6 clones. 19 V_1^R; 14 V_1^R

1 C 7 clones 30, 29.

D 1? clone. 12, 12

A29. Pool pickings ~~from~~ homologous sites and restreak on EMB Lac. save original cultures Transfer to plain, T1 agar.

2 A 15? clones; ca 25 mutants Restreak from 5 sites

B 13? " ca 40 " " " " (without pooling)

C 12 " all v thick streaks 18 muts.

D 1 21

A3-4. 14 clones; 37 mutants. Pool from 5 sites into ca. 1 ml.
Plate serially to further dilutions.
Suspend B, C experiment.
D transfer to plain; 2 T1. 7/3/51. Hold in refrig.

7/4/51. ~~Transfer D3~~
D3: 3-4 clones (?) ca 20 mutants.
Plate as #4:

A4. Transfer to series of T1 plates at 1, 3, 6" spreading

****

This procedure is obviously not working properly, presumably because of confusion between mutants within an inoculum to a plate, and those which occur during the growth of colonies on the plate. The samples from homologous sites are probably too small to have a reasonable probability of carrying an identified clone to the next inoculum.

The procedure should be modified as follows: A broth culture should be spread or streaked on plain agar, and permitted to grow for just a few generations. The thinly grown plate should then be printed to selective and plain agar, and the clones identified. The homologous site should then be inoculated to a small volume of broth, which is then restreaked in the same manner.

Lee Adelberg
Fed. Proc. 1952

Aberrant Linkage

June 26, 1951

A W1177 × 1632 EMS Lac.

B W1635 × 1632

Count from plates: (Lac)

A.	+	−		B	+	−
	17	30			187	18
	2	27				
	13	33				
	14	26				
	46	116	162			

"B" B Lac+ } selections
"C" B Lac− }

Picks A "at random" to EMS Lac. B 100+ 48−

Paint ~~Transfer~~ to EMS Lac, Mal, MTL, sm; T1 for sensitivity

	Lac	Mal	S	MTL	T1	Gal	Xyl
A. 1	−	−	R	−	S	−	−
2	+	−	R	−	S	−	−
3	−	−	R	−	R	−	−
4	−	−	R	−	S	−	−
5	+	−	R	−	S	−	−
6	−	−	R	−	S	−	−
7	−	−	R	−	R	−	−
8	−	−	R	−	R	−	−
9	−	−	R	−	S	−	−
10	−	−	R	−	R	−	−
11	−	−	R	−	S	−	−
12	−	−	R	−	S	−	−
13	−	−	R	−	R	−	−
14	−	−	R	−	S	−	−
15	−	−	R	−	R	−	−
16	−	−	R	−	R	−	−
17	−	−	R	−	R	−	−
18	+	+	S	+	S	+	+
19	−	−	R	−	S	−	−
20	−	−	R	−	S	−	−
21	+	+	R	+	S	+	+
22	−	−	R	−	S	−	−
23	+	−	R	−	S	−	−
24	+	−	R	−	S	−	−
25	+	+	S	+	S	+	+
26	−	−	R	−	S	−	−
27	+	+	S	+	S	+	+
28	−	−	R	−	S	+	−
29	+	−	R	−	S	−	−
30	+	−	R	−	S	−	−

A

	Lac	Mal	S	Mtl	T1	Gal	Xyl
31	+	−	R	−	S	−	−
32	+	+	S	+	S	+	+
3	−	+	R	−	R	−	−
4	−	−	R	−	S	−	−
5	+	−	S	−	S	−	−
6	+	−	R	−	S	−	+
7	−	−	R	−	R	−	+
8	+	−	R	−	S	−	−
9	−	−	R	−	S	−	−
40	+	−	R	−	S	−	−
41	+	+	R	−	S	−	−
2	−	+	R	−	S	−	−
3	−	+	R	−	S	−	−
4	−	−	R	+	R	−	+
5	−	−	R	−	R	−	−
6	−	−	R	−	R	−	−
7	−	−	R	−	S	−	−
8	−	−	R	−	S	−	−
9	+	−	R	−	S	−	−
50	+	+	S	+	S	+	+
51	+	−	R	+	S	−	−
2	−	−	R	−	S	−	−
3	+	+	S	+	S	+	+
4	+	−	R	−	S	−	−
5	+	+	S	+	S	+	+
6	−	−	R	−	S	−	−
7	−	+	S	+	S	−	+
8	−	−	R	−	S	−	−
9	−	−	R	−	S	−	−
60	+	−	R	−	R	−	−
61	−	−	R	−	R	−	−
2	−	−	R	−	S	−	−
3	+	−	R	+	R	−	+
4	+	+	R	+	S	−	+
5	+	+	S	+	S	+	+
6	−	−	R	−	S	−	−
7	−	−	R	−	S	−	−
8	−	−	S	−	R	+	−
9	+	+	S	+	S	+	+
70	−	−	R	−	S	−	−
71	+						
2	+						
3	+						
4	+						
5	+						
6	+						

	Lac	Mal	S	MR	TI	Gal	Xyl
71	+	−	R	−	S	−	−
72	+	+	S	+	R	+	+
3	+	−	R	−	S	+	−
4	+	−	S	+	S	+	+
5	+	−	R	−	R	−	−
6	+	+	S	−	R	−	−
7	+	+	S	+	S	+	+
8	−	−	R	−	R	−	−
9	+	+	S	+	S	+	+
80	+	−	R	−	S	−	−
81	+	−	R	−	S	−	−
2	−	−	R	−	S	−	−
3	+	+	S	+	S	+	+
4	+	+	S	+	S	+	+
5	−	−	R	+	R	−	+
6	−	−	S	−	S	−	−
7	−	−	R	−	R	−	−
8	−+	−+	R	−+		−	−
9	−	−	R	−		−	−
90	+	+	R	−		+	+
11	−	+		−	R	−	−
2	−	−		−	S	−	−
3	+	+	S	+		+	+
4	+	+	S	−		+	−
5	+	+	S	+		+	+
6	+	−	R	−		+	−
7	+	−	R	−		−	−
8	−	−	R	−	R	−	−
94	+	+	S	−		+	−
100	+	+	S	+		+	+

=Lac+ selectants

852B

B2

	lac	Mal	Mtl	S	TI	Xyl	Gal
1	+	(−)	+	R		+	+
2		(−)	+	R		+	
3		(	+			+	
4		+	+	R		(−)	
5		+	+	R		+	
6		+	+	R		—	
7		+	—			—	
8		(−)	+	R		+	
9		+	+			+	
10		(−)	—	R		—	
11		—	—	R		—	
12		(+)	+	R	R	+	
13		+	+		R	+	
14		+				+	
15		+	—			+	
16		+				+	
17		+	—			—	
18		+	—			+	
19		+				+	
20		—	—	R		+	
21		+	+			+	
22		+	+				
23		+	+				
24		—	—				
25		(+)	+	R			
26		+	+				
27		+	+			=	
28		+	+			+	
29		+	+		R		
30		+	+			+	
31		+	+			—	
32		+	+	R		+	
33		+	+			—	
34		—	+	R		+	
35		+	+			+	
36		+	+	R		+	
37		+	+			+	
38		+	+			+	
39		+	+			+	
40		+	(+)	R		—	
41		+	+			—	
42		+	+			+	
43		+	+			+	
44		+	—			+	
45		+	+			+	
46		+				+	
47		+				+	
48		+			R	+	
49		+			R	+	
50		+	+	R		+	

B1

	lac	Mal	Mtl	S	TI	X	Gal
51	+	+	+		R	+	+
52		+	+			+	
53		+	+			+	
54		+	+			+	
55		—	+	R		+	
56		+	+	R		+	
57		+	+			+	
58		+	+			+	
59		+	+			+	
60		+	+			+	
61		+	+			+	
62		+	+	R		+	
63		+	+	R		+	
64		+	+			—	
65		+	+	R		—	
66		+	+			+	
67		+	+			+	
68		+	+			+	
69		+	+			+	
70		+	+	R		+	
71		—	+			+	
72		+	+			+	
73		+	+			+	
74		+	+			+	
75		+	—			—	
76		+	+	R		+	
77		+	+			+	
78		+	—			+	
79		+	+			+	
80		—	+	R	R	+	
81		+	+	S		+	
82		+	—	S		—	
83		+	+	S		+	
84		+	+	R	R	+	
85		—	—	S		—	
86		+	+	S		+	
87		+	+	R		+	
88		+	—	S		—	
89		+	+	R		+	
90		+	+	S		+	
91		+	+			+	
92		+	+			+	
93		—	+			+	
94		+	+	R		+	
95		+	+	R		+	
96		+	—			+	
97		+	—			—	
98		+	+			+	
99		+	+			+	
100		+	(+)			+	

Repeat M- segregation
W1111 x W1632.

July 17, 1951.

Cross on D: MBB, sm) gar.
Test "protoplasts" by plate transfer to D: BB, sm)
Tests very clear.

Plate	M-	Total
1	8	101
2	1	27
3	4	84
4	2	85
5	5	87
	20	384

Also pick 20 at random for M+. Purify on EMB Lac.
Pick to EMB.

M-	7 Lac+	13 Lac -	
M+	3 +	11 -	

not significantly different.
M- grew very well on plating media.

Isolates: M- all grew on BMS, not BB, ✓.
M+ " " " ✓

on EMB Maltose	M-	3 Mal+ (absolute)	others -
	M+		all Mal -

Selection, possible lac −

852 C

	Lac	Mal	S	Mtl	TI	Gal	Xyl
1	−	+		−		+	
2	+	+		+	R	+	
3	−	+		+	R	+	
4	+	+	R	+		+	
5	−	+	R	+	R	+	
6	−	+		+		+	
7	−	+	R	+	R	+	
8	−	+		+		+	
9	−	+		+		+	
10	−	+		+		+	
11	−	+		+		+	
2	+	+		+		+	
3	−	+		−		+	
4	+	+	R	+		+	
5	−	+		+	R	+	
6	+	−	R	−	R	+	
7	−	−	R	+		+	
8	−	+		+		−	
9	−	+		+		+	
20	−	+		+		+	
21	−	+		+	R	+	
2	+	+	R	+	R	+	
3	−	+		−	R	+	
4	+	+	R	+		+	
5	+	+		+		+	
6	−	−	R	−	R	+	
7	−	+	R	+		+	
8	−	+		+		+	
9	−	+		+		+	
30	+	+	R	+		+	
31	−	−		−		+	
2	+	+		+		+	
3	−	+		+		+	
4	+	+		+		+	
5	−	+	R	−		+	
6	−	+		+		+	
7	+	+	R	+		+	
8	−	+		+		+	
9	+	+	R	+		+	
40	−	+		+	R	+	
41	+	+	R	+		+	
2	+	+		+	R	+	
3	−	+		+	R	+	
4	+	+		+	R	+	
5	−	+		+		+	
6	+	+	R	+		+	
7	−	+	R	+	R	+	
48	+	+		+	R	+	

Counted 852C Lac-mly.

852C'

Mal	S	MR	TI
+	S	−	S
+	S	+	R
+	R	+	R
+	S	+	S
+	S	+	S
+	S	+	S
+	S	+	S
+	S	−	S
+	S	+	R
−	R	+	S
−	R	−	R
−	S	−	S
+	S	+	S
+	R	−	S
+	S	+	S
+	S	+	S
+	S	+	S
+	S	+	R
+	S	+	R

Selected isolations

852
D

June 29, 1951.

Cross B EMS lac. Print 3 plates to EMS lac-, Mal, MH for specific selections. #1, 2 rather smeared (use better support maybe)

#3 OK.

Pick all Mal- and MH- lac- from #3 plates (including overlaps).

Count. Lac: 10- 163 + / 173

Mal ~~29~~ 30 } 7? overlaps
MH 21

Lac-MH 1? ov.
lac-Mal 0?

S^R : 27 : 8 Mal+
19 Mal-

From the interaction of the - Lac, MH, Mal selections, in comparison with unselected data of B, useful information on linkage relations may be obtained.

Summary

Lac	Mal	S	Mtl	T1		A	B	Adj	
−	+	R	+	R			/	1	1
−	+	R	+	S	1	1 /			
−	+	R	−	R	3	2 //			
−	+	R	−	S	4	3 ///	/	1	1
−	+	S	+	R			////	4	3
−	+	S	+	S			~~////~~ ///	7	6
−	+	S	−	R					
−	+	S	−	S			//	2	2
−	−	R	+	R	3	2 //			
−	−	R	+	S			/	1	1
−	−	R	−	R	22	12 ~~////~~ ~~////~~ ~~////~~ /	/	1	1
−	−	R	−	S	36	26 ~~////~~ ~~////~~ ~~////~~ ~~////~~ ~~////~~ /			15
−	−	S	+	R					
−	−	S	+	S					
−	−	S	−	R	1	1 /			
−	−	S	−	S	2	1 /	/		
+	+	R	+	R			//	2	2
+	+	R	+	S	1	2 //	~~////~~ ~~////~~ ~~////~~ /	16 13	13
+	+	R	−	R					
+	+	R	−	S	1	2 //			
+	+	S	+	R	1	1 /	~~////~~	5	4 5
+	+	S	+	S	9	16 ~~////~~ ~~////~~ ~~////~~ /	~~////~~ ~~////~~ ~~////~~ ~~////~~ ~~////~~ ~~////~~		5
+	+	S	−	R	1	1 /			
+	+	S	−	S	1	2 //	~~////~~ ///	8	7
+	−	R	+	R	1	1 /	/	1	
+	−	R	+	S	1	1 /	~~////~~ /	6	5
+	−	R	−	R	1	1 ~~////~~ /			
+	−	R	−	S	11	19 ~~////~~ ~~////~~ ~~////~~ ////	///	3	
+	−	S	+	R					
+	−	S	+	S	1	1 /	//	2	
+	−	S	−	R					
+	−	S	−	S	1	1 /	///	3	

A

~~////~~ ~~////~~ ~~////~~ ~~////~~ ////

852

Adjusted (to 100)

Summary used in CSH 1951

	Lac	Mal	S	MH	T1		
	−	+	R	+	R		1
	−	+	R	+	S	1	
	−	+	R	−	R	3	
	−	+	R	−	S	4	1
	−	+	S	+	R		3
	−	+	S	+	S		6
	−	+	S	−	R		
	−	+	S	−	S		2
	−	−	R	+	R	3	
	−	−	R	+	S		1
*	−	−	R	−	R	22	1
	−	−	R	−	S	36	
	−	−	S	+	R		
	−	−	S	+	S		
	−	−	S	−	R	1	
	−	−	S	−	S	2	
	+	+	R	+	R		2
	+	+	R	+	S	1	13
	+	+	R	−	R		
	+	+	R	−	S	1	
	+	+	S	+	R	1	4
*	+	+	S	+	S	9	45
	+	+	S	−	R	1	
	+	+	S	−	S		7
	+	−	R	+	R	1	1
	+	−	R	+	S	1	5
	+	−	R	−	R	1	
	+	−	R	−	S	11	3
	+	−	S	+	R		
	+	−	S	+	S		2
	+	−	S	−	R		
	+	−	S	−	S	1	3
A	%+S	28	21	15	17	67	
B	"	85	84	72	73	88	

Segregation of M−

July 3, 1951.

A W1177 × 1632
B W1635 × 1632

Cross on 1. D(0) + BMB$_1$ sm for segregation of M+/−

July 7. B' B, grown together 1.5 hours 2. EMS Lac for linkage data. on D(0)

July 3: A: numerous colonies on BxB, sm.
B 1 " 6 plates!

Repeat B, B'. July 6.

A. Test, by velvet transfer, M segregation from A.
Not all scoreable.

3. 83/83 M+
2. 7/48 M− } 12 M− ✓
1. 5/62 M− }

also recover 12 M+ ✓) 14 ± —.

B' 9 colonies. Test on +, − M.

all are M+. ∴ M is + [illegible] Th
TL
−
S −.

Suggestion: In "B", is Lac linked to TL?
Test by crossing BM S^R × TL s^s
on TL sm agar
and studying segregation
of TL, Lac.

(see over)

Lac character of M+, M- selections

B'	M+	6 Lac+	2 Lac-
A	M+	12: 3 Lac+	9 Lac-
	M-	11: 2 Lac+	9 Lac-

Linkage of Lac to M? Not supported by these data.

Lac- mutants in W1634

July 6, 1951.

W1634 (Cellobiose-fermenter). Irradiated 8 secs on EMB Lac
2~~0~~5 x 50 = 1~~0~~250 colonies.
Some sectorials undoubtedly ignored. Many morphological sectored cols.

Spot Lac- on EMB Glu for further purifying.
By ~~transfer~~ print tests:
13 Lac- all are Sucrose Cellobiose Xylose Galactose Maltose and glucose positive

Save 1. Lac- 854 1

July 12, 1951.
20 x 50 = 1000 on EMB glucose No definite mutants
15 x 40 = 600 " " " " " "

7/16. Check Vaughn's cultures 776-835, 837, 839, 840, 841 1-5
s.o., EMB glu, cell, suc, lac
7/17. all are ++ or +. #1,3 probably most generally suitable
= 835 (Vaughn 29) = W 1647
= 839 (Vaughn 168) = W 1648

7/17. a 1634
b 1647 — uv resistant
c 1648
854-(2-4)
a. 3 possible Glu- from 12 x 400 = ca 5000 ✓
c 1 " " " 8 x 100 = 800. ✓ (+ second very well!)
= 854-5
All 4 mutants grow rather poorly. Are negative on galactose, glucose, xylose, mannitol, maltose, lactose, sucrose and cellobiose

see over

(1648??)

W1647. UV

1 Glu- mut on 5 plates, variably recorded

? Lac- or ±

(1). Glu- Cb- Suc + Mal-Lac-
Xyl+ Gal+

Designation of W1647 almost certainly correct, but conceivably was substituted for W1648. Designate 1647a for immediate source of Glu- mutant (W1677)

Penicillin effects on K12

July 8, 1951.

24 hr. K12 culture .5/10 ml fresh Pennassay + penicillin as indicated.

1. No penicillin
2 1 unit/ml
3 5 units/ml

* P9. Filter 1, then 2; 3 then W1177 (4) as controls.

	1	2	3	4
A. Plate .1 ml on EMB lac	✓st	✓~~st~~ 1+	1 col. lac+	
B. " .1 ml with W1177 on ~~E~~ ~~B~~ EMS lac			2 minute lac-	
C. Inoc. 10 ml Pennassay	✓st	✓st	Turbid	Turbid
D. " " + W1177.	st	1 minute lac-, r? streak out		

AA etc. same but N10., (~~W1177~~) probable leaks

	1	2	3
AA	++	++	
BB (W677)	+0	+?	
CC	2 lac+		st.
DD	7 lac+	7 lac+	st

Possible "prototrophs"? in 3B, 2D
Restreak in D (0)

This experiment n.g.:
leaking gasket on filter
used for 1 and 2

With 1-5 units, no morphological effects were noted.

Penicillin effects.

July 11, 1951.

P12. P12.

K12. 1 ml + A Penicillin in Penassay tubes ~~++++~~
B

1	A	0	++	
2	B	50	++	Normal size + motility.
3	C	50	++	many filaments. some minute granules
4.	D	120 u/ml	±	inhibited growth.

Filter 1, 2, 3 through separate 8-9 lb Mandler filters. Pass turbid (ca 10^8) W1177 broth culture each bulk sterility. Plate this filtrate on EMB Lac (= E) and inoculate 1 ml into Penassay (= F).

		1	2	3
A.	Inoculate filtrates 1 ml, Penassay	—	—	K12 only ++
B	" " " " + W1177. Plate after 24.	0	0	~~++~~ K12
C	Plate " .1 " EMB Lac	0	0	0
D	" " EMB Lac + W1177	0	0	0
E		0	0	0
F		—	K12 only ++	K12 only ++

A few particles per ml ~~survive~~ survive filtration, if penicillin has been added. No unique genetic activity is noted. However, higher titres of FA, and heat-inactivation are needed to parallel Salmonella findings. W1177 control satisfactory.

Also plate filtrates into serum agar (+ penicillin ?)

F: streak out on EMB lac + or − see to find W1177.

75u Penicillin K12 1% inoculum. 4½. 37°
" R.T.

A. Sediment and filter supernatant. 14 under 81b filter
B. Follow with untreated W1177 >1% (.01/ml) control.

---

1. Plate A on serum agar; 0.1ml
2 " " nutrient " "
3 " " EMBlac 0.1 "
4 Incr 1ml samples into Penassay.
5 " " " + .5ml W1177
6 Plate B on EMB Lac sen
7 Incr 1ml Penassay.

Conclusion: Filter not satisfactory. 6 showed ca 50 lac-
1,2,3 each showed ca 400 500 (lac+). 4 all turbid.

Refilter unirradiated suspension, 141b Mandler hypothesis all sterile. No protoplasts from 5, 7

Conclusion: Penicillin may result in slight augmentation of lethality of K-12, but no consistent effect noted. No genetic effects whatever noted.

5. showed minute colonies on EMS lac after 3-5 days

Pick and streak out on EMB ~~S~~ lac, D/O). – 3 days: no growth on both

[Probably T-L+ revertants growing on carryover]. not + on former.

8 hour W1649 + 855b Inc'd in Primary.

9 " W1632 " " " "

Wash + plate heavily EMS lac

No colonies

Conclusion: No evidence of genetic activity in penicillin treatments of K12.

TL- +-1 in linkage orientation

- F2 Crosses

July 6, 1951.

Cross D TLB, sm W677 x

A W1367 No yield ~~Sm 88 T~~
B W1302 No yield.
C W1490 2 colonies / 2 plates — 1 grew out: B_1 —

D W1368 x W677

D' (do., grown together 6-8 hours).

7/17 D: Transfer plates to D (sm TLB$_1$);; EMS Mal sm TB$_1$.
Only 1 out of about 60 grown on EMB B$_1$.

D' ~~Most grew on EMB B$_1$, few on TLB$_1$ (results mix up of plates)~~

↓ Streak out auxotrophs on EMB Lac to purify for further characterization. 8 saved.

#1 ~ T-L-B$_1$ - S^R Lac- W1649.

~ all 8 are T-L-(B$_1$-) S^R Lac- Mal- Xyl- Mtl- Sal- (S^R Mutant ??)

7/19/51. E. W1649 x ~~W1368~~ W1632 on EMS Lac, Mal, Mtl.

EMS Lac 95%+
Mal " +
Mtl 90%+.

∴ Haploid strict recombinants also give aberrant linkage results.

Alternate page

Material from 853 W1635 x W1632.
852

EMSMR 41- 359 ~~total~~

Strokes from D10) to EMS Lac.
Also collect Lac- from EMS Lac plates

Repurify all Lac-.

Plate count
EMS Lac. 18 - : 187+
MR 9 - ~~55~~ 64 ~~total~~ 160
From D10) strokes to EMS Lac 13/4 - : 245 total

Read prints from Lac+ and from Lac - separately.

a. Transfer all Lac+ to MR, Mal.

b. Transfer 50 Lac+ also to Xyl, T6, T1

c. Transfer 28 Lac- to MR Mal T6 T1 Xyl

3/16/52 Conclusions?: Lac, Mal are independent

(1)

✓Malon EMB

LAC+	Lac	Mal	Mtl	EMB Xyl	S	T1	T6
1			–	–		R	R
2			–	–			R
3							R
4							R
5		–		±	R –		R
6			–	–	R –	R	R
7							R
8							R
9		–	–	±	R –		R
10			–	–			R
11							25 R
12							26 R
13					R ✓+		27 R
14							28 R
15			–				29 R
16			–	–			30 R
17					R –		31 R
18							32 R
19							33 R
20							34 R
21						R	3 R
22	–						
23	–	–	–(+)	±	R –		
24				–			35 R
25		–		–			36 R
26		–	–				
28			–				
30			–				
31			–	–			
33		–	–	–	R –		
36		–	–	–	R –		
40				±	R –		
41			±		R –		
43		∓	–	– (+)?	R –		
45				–			
46			–				

only few Lac- V6 [illegible]

ALL EXCEPT #13 /MAL–

	LAC	MAL	MTL	EMB XYL	EMS LAC S	T_1	T_6
51							
52			-				
53		-	-	±	R -	R :	R
54					R (s)-	R :	s ✓
55	-	±				~~R~~	
56							
57							
58							
59							
60							
61					R ✓+		
62	+	-	?	-			
63		-			R -		
64							
65							
66		-				R :	R
67		-	-	-	R -		
68							
69			-	-			
70					R ✓+		
71			-	-			
72							
73							
74				-		R mvc	
75	-		+ MUCOID			R ~~lac -~~	
76		-	-	-	R -		
77			-	-			
78			-				
79						~~R~~	
80	-						
81	?					R :	R
82							
83		-	-	-	R -		
84	+						
85			-	-+			
86						R :	R
87							
88							
89							
90			-	-	R -		
91			-				
92							
93							
94		-	-	-			
95	-		-	-			
96							
97							
98							
99							
100			-		R -		

3

	LAC	MAL	MTL	XyL	EMB S	EMS LAC T1	T6
101							
102					R +		
103	−					(R)	
104							
105							
106							
107							
108							
109				+			
110							
111							
112							
113							
114			−			(Mucoid R)	
115							
116							
117							
118			−	−			
119							
120							
121							
122							
123					R +		
124							
125							
126							
127			−+	−+			
128							
129							
130							
131		−					
132							
133			−				
134							
135		−	−	−	R −		
136		−	−	−	R −		
137							
138							
139							
140							
141							
142						R .. R	
143							
144							
145							
146	−						
147		−					
148				±			
149							
150					R +	R . R	

4

	LAC	MAL	MTL	XYL	CMS LAC S	T_1	T_6
101							
152		−	∓	±			
153							
154			∓	−			
155						R	R
156			∓				
157							
158		−+			R^+		
159							
160		−					
161			−	−	R^+		
162							
163						R^{Lac-}	
164							
165			−	−+			
166							
167							
168	−+						
169							
170	−	−+				R^{Lac-}	
171			−	−		R	R
172			−	−			
173							
174							
175			−+	−+			
176							
177	●						
178							
179							
180			−	−+			
181							
182							
183							
184							
185		+	−	∓			
186							
187	∓			∓	R^{+Lac-}		
188			−	−			
189							
190						R	R
191							
192							
193		−	−	−	R^-		
194						R	S
195							
196							
197							
198	∓						
199							
200			−	−			

	LAC	MAL	MTL	XYL	EMS LAC S	T_1	T_6
201							
202		∓			R⁻		
203			−	∓			
204							
205	− ✓					R	
206					R⁻		
207							
208							
209			−	∓			
210							
211			−	−			
212		−	−	−			
213							
214							
215						R Lac−	
216			−	−			
217	∓ ✓						
218							
219					R⁻		
220							
221							
222					R⁻	R	R
223		∓			R⁻		
224							
225							
226							
227						R Lac+	
228							
229		−	∓		R⁻	R	R
230					R⁺		
231					R⁺		
232							
233		−			R⁻		
234							
235							
236							
237			−				
238					R⁺		
239		−	−	− (+)	R⁺		
240	∓ ✓				R⁻	R Lac−	
241							
242							
243					R⁻	R Lac−	
244							
245							
246							
247							
248							
249							
250							

	LAC	MAL	MTL	XYL	S	T_1	T_6	
1						R	S	37
2			–	–	R MAL+		S	
3						R	S	
4						R	S	
5							S	
6							R	42
7						R	S	
8							S	
9							S	
10							S	
11						R R	S	4
12						R	S	
13							S	
14		–	–	–			S	
15		–					S	
16		–	–	–	R –		S	52
17							S	
18							R	
19						R	S	
20			–	–			S S	6
21					R MAL+		R	
22						R	S	
23						R	R	
24						R	S	
25							S	
26		–	–	–	R –		S	
27			–	–			S	
28							R	

Lac+

Lac –

July 10, 1951

P10 1. Inoc. 1 colony N-1 ē Penassay., NSA slant
a b
~~[struck out]~~ Spread .1 and .01 ml on EMB lac. Assay .1 ml ē T1.
9[30] A12

4P Print to I T1 again.

A 13: Read:
a 46 clones; 31 singles; (1 plate); 21 singles (second plate).
b. 10 clones; 6 singles (on plate only)

Pick from b sites ē fresh broth.
Spread .001 ml ; streak. Grow 4 PM - 10[30] PM
Print A 10[00] PM. B.

AM .001 ml: 15 clones 10 singles (1)
5 " (2)

(3) Pick 5 sites.

N15 Plate at various dilutions
5 hours incubation:
Print to T1.

– Assays of successive isolates.
1 10^{-6}/ml 91,5 mnc.
2 5×10^{-6} 1 ml ca 500.
3 10^{-6} .01 ml ca 1000.
4 1×10^{-6} 38/562

A streak : 1 clone in nearly confluent portion. Restreak directly, also testing and compare cross-brush with T1, as against random isolated colonies. Pick 1 clone = 1

B 10^{-5} Semiconfluent. 9 clones; no singles.
Pick 3 clones = 2-4 streakout
pool to Penassay.

See over:

1. Random tests on streaked colonies. / T1.

1.	10 S.	0 R
2.	"	"
3.	25 S	5 R.
4.	8 S	2 R

∴ successful isolation of V_1^R by indirect selection

2. Dilute 858-4 10^{-6} plate. 1 ml. 3 plates.

#2 smeared - too wet

Permit to T1 after 9 hours (pinpoint colonies)

1.	12 V_1^R	301	total
3.	16	261	"
	38 /	562	

Pick best isolated homologous colonies. Test against T1 and streak to purify.

✓ each of 4 V_1^R

2 random V_1^S.

Store single colony as 858-5 ... grow + transfer broth to test stability

Note In final plating a few plaques of contaminant phage (identified as T1) were found. However, no evidence of T1 was found in the broth series, or in selection line. It seems unlikely that this could interfere with achieved result. However, see 859 for repetition of general expt.

Indirect Selection: W-112 / T1.

July 17, 1951.

Initiate single colony broth cultures 11 AM. = 859-1

P17 Plate EMB lac, Incubate ca 5 hours. Print to T1 plates .01 ml

5 clones; Total: 16; 12

N18. Picks clone sites to Penassay.

3-8 PM. $^{A}10^{-2}$, 10^{-3}, 10^{-4} ml plated on EMB lac

8 PM Print to T1 plates

A19. A. 23 clones, 1-3 singles.

B 1 clone 2 singles

C 1 clone 1 single.

Pool clones from B and C to Penassay. 859-3

N19. 2 hour tube probably ca 3×10^8) plated out on EMB lac

12 N19 — 10^{-3} A, 10^{-4} B, 10^{-5} C

q^{30} A20. C: (ca 600 cells) No V_1^R

B: 2 clones; 1, 0 singles

A: 16 clones; 2, 0 singles.

Pool 2 sites from B and 2 from A to Penassay 859-4.

10 A21. 10^{-6} dilution: 3 plates. After 4 hours, make replica transfer.

A

B 12 mutants; 793 Total

C

Streak out 4 of the V_1^R.

Test against T1. V_1^R ✓

Place associated V_1^R on slant.

Initiate serial transfers in broth, 5A, B, ...

858 and 859. Each transfused serially through 10 5ml tubes Penassay with loopful inocula.

8/3/51. Plate out on EMB glu. Replica to EMBLac. T1. all colonies are V_1^R. W1485 control lysed on T1 plates.

Total counts:

858	859.
290	131
277	150
269	166
836	447

Replica Efficiency

July 24, 1951.

A. Compare fresh and 4 day broth for clones.

1. Dilute 58-161 in EMB (4 ml) 9 AM.
2. Spread " " " " 315 PM.

325. Replica to T1 agar, pairs.

B. Dilute 58-161 10^{-5} Spread .1 ml, let dry.

① Replica to series of plain agar.

② Assay 10^{-7} ml for count.

③ As ①, but 10^{-5} ml originally

			(Replica removed) ↓				
(B) 2:	10^{-7}	0	1	2	3	[illegible]	
		99	91	7	1		
1	10^{-6}	(1000)	± 10^3	42	19	12	10	6
3	10^{-5}	(10,000)	± ca 10^4	331	171	108	109	87

∴ About 10-25% of initial cells are removed.

5-10% deposited on first replica, with indicated "decay" thereafter.

8/30/61 — see experiments with E. Klein on efficiency of colony transfer,,

Singlets and clones
Replica plating

July 25, 1951.

2¹⁵ P.M. W112 (859-1)

1a. 1 ml + T1 assay 190
b. 02 ml + T1 assay. 90 ~~78~~

2a ~~.02 ml~~ .1 ml Replica to 2 T1 plates immediately
b .02 " " " "

3a .1 " " after incubation 2¹⁵ -
b .02 " "

2a	15	4	0	) no sign of clones.
2b	2	0	6	
3a	73	65	53	many clones.
3b	14	10	5	
2bb	11	8	4,	2nd Replica suis from 2b after incubation.

Use 2bb and 3b for published figures of replicated clones

7/27. Nonclonal replicas.
c1 .1 ml W-1 culture transfer (EMB) as initial
c2 1 ml (sed + conc to .1 ml ⇒ 10:1)

Primary segregation ratio: H226.

July 28, 1951.

Use technique of (respreading microcolony)

2-wk old culture H226. 10^{-6} dil.

Control: .1 ml spread over plate; .01 ml in line

Expt'l. .01 ml in line 11:30 AM. Respread after 7½ hours

mostly segregated for N.S. 7 PM.

EMB Lac.

Repeat, new H226.

7/29. Mostly segregated. By replica, almost all Lac- are prototrophs. 1 aux. Lac- noted = 862-1 see 868

7/30. New H226. → (Lyophil) from nigle colony. 10 AM. – 3 PM = 5 hours. 10^{-6} .01 ml.

Control plating .1 ml: 166 23

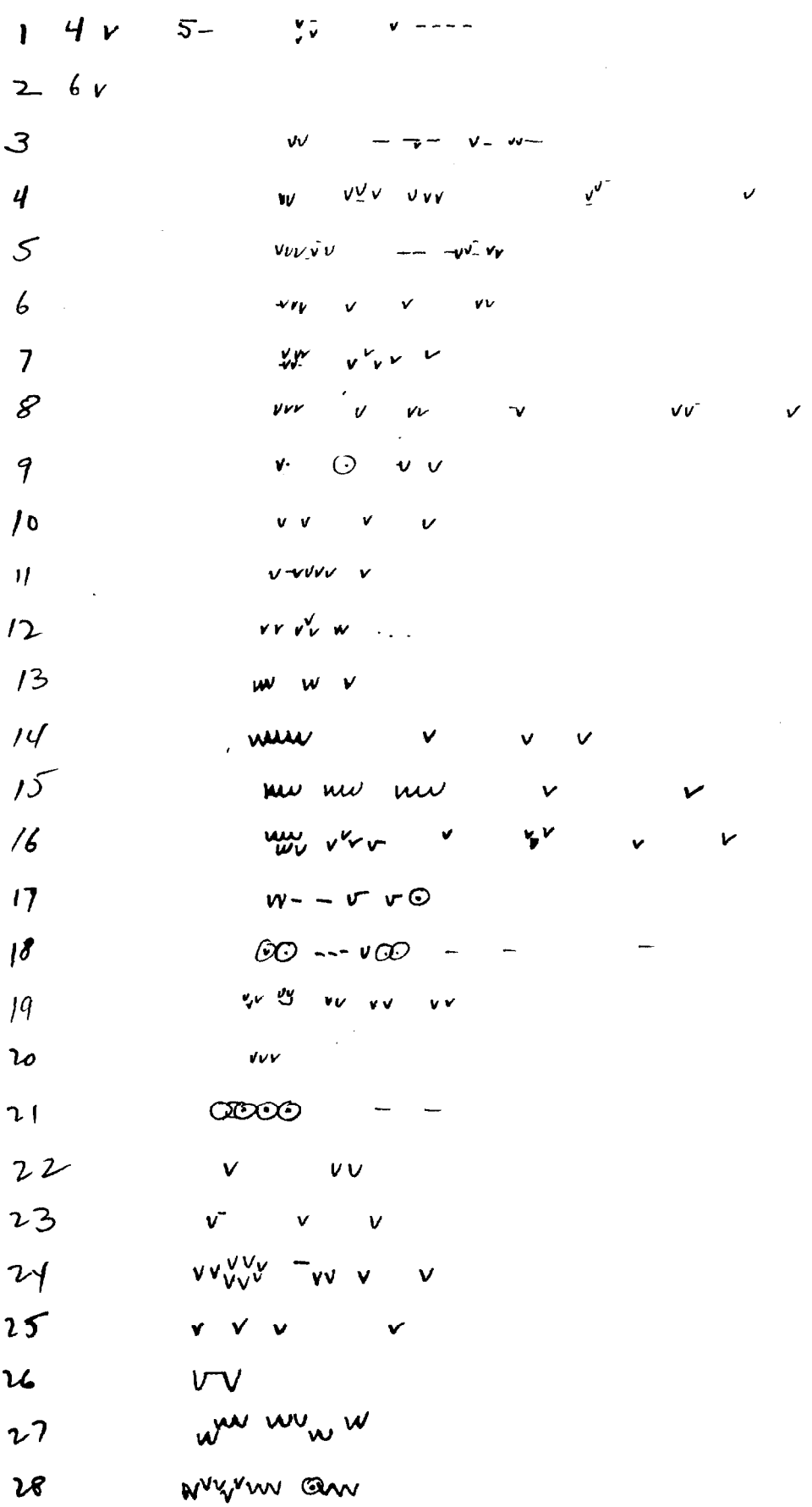
.01 ml brush. 29 4

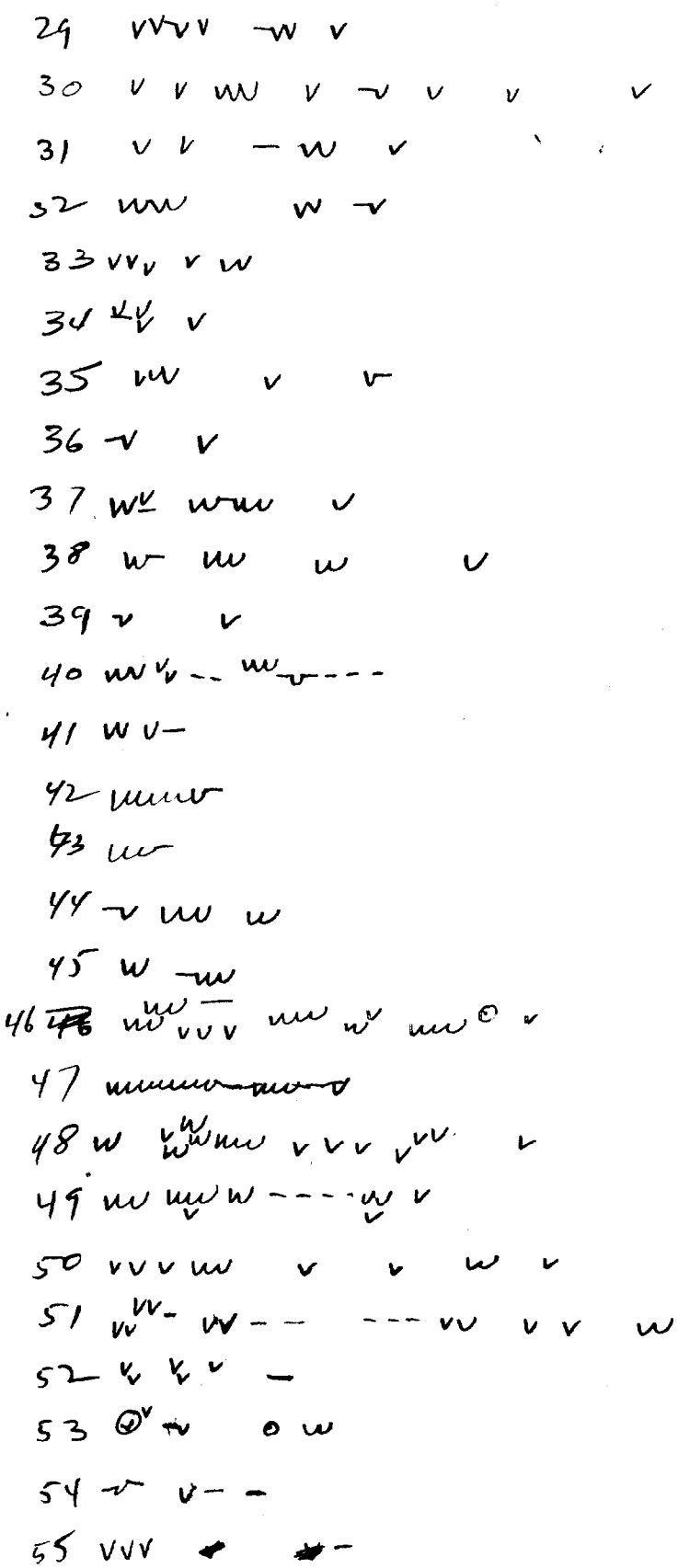
Probably 2× too many cells per brush, but many likely

From replica plates, most Lac- are auxotrophic;

Replica Lac- "segregant" solutions to EMS Lac

Clones





56 V V V V V

57 V V V V V V

58 V V ⊙ V

59 ⊙ V

— clones

15, 7, 24, 10, 17, 16, 15, 21, 19, 21, 14

many unique clones. Pick possible new — from lacV clones as best possible. Pick to EMB Mal

---

all picked were Mal+. #52 MalV, prototrophic all others auxotrophic.

all Xgl+ except 29, 57.

Most likely most of the Lac− were previous segregants

see 867

W. K. Maas cultures
Inter-sterility.

July 30, 1951

A. KIGT-h- } pts.
B. KI h2-arg }

Add put to all plates, EMS Lac
1 plate per x.

1. A x W-1
2. B x W-1
3. A x H245
4. B x H245
5. A x H290
6. A x W1606 (es BM-)

KI GT-h-
= put-multicam-hist-

KI h2-arg
= put hist arg.

8/2/51. all plates barren. Other crosses with W1606, H245, H290 were fully fertile.

Maas says put+ mutations are futile.

July 29, 1951.

a. Irradiate on EMB agar 6-20 secs.

b. Irradiate washed suspension 2 ... 20 secs. Drop ½ ml into Penassay. Use 4 sec treatment

7/30 Plate on EMB Lac

Ca 300 tested for auxotrophy by replica: all X+.

No Lac, or Mal variants on ca 20 plates. Many colonies suggesting morphological characteristics.

ca 300 more tests for auxotroph. ~~I doubtful: Retests~~

10 x 350 = 3500. No auxotrophs detected!

Total, ca. 4000 tests!

Plate on EMB Mal sm. [E. Woodworth records show W1647.]

Variable types, some Mal ±. All Lac+.

1
2
3

9/20/51. Problem adopted by E. Cohn.

W1606 × H293 ; W1675 × H290
S^D × S^R

865

Mostly lac+. Replica to EMSlac, EMSlac sm. for S^S.
ca 200 colonies — grew exquisitely on
EMS Lac, ± Sm.

Aug. 4, 1950 ~~W1606 ×~~
B W1675 × H290.

C. W1675 × H294 on EMSlac, ± sm
TL lac-Mal-S^D M-lac+Mal+S^S
8/5/51

"1675" proved to be BM- lac+Mal+S^D
(probably W1606)

Losses n.g.

July 30, 1951.

M290 × M293

a. EMS Lac b. EMS Mal

M- Lac v Mal- S^S TTL- lac v Malt S^R

a. 20 picked. 11 Lac v. 9 thus lac- (Lac v.)

b " " 8 Mal-
~~8~~ 7 "+"
3 Mal v
2 ?

Test ~~[redacted]~~ single colonies on EMS Lac; EMB Mal / sucr.

~~[redacted]~~:

	Mal	S	#
b.	—	S	8
	+	R	5
	~~4~~ v	V	2?
	+	S	4
a.	—	S	9
	+	R	1

Mal v relatively infrequent in this cross.

Grow 866-1 on D(Lac) for mucoid patches on EMS Mal.

Diploid microcolony segregations

August 2, 1951.

See 862 .01 ml; $.3 \times 10^{-6}$ per plate $11\frac{30}{}$ -

assay .1 ml EMB Lac

V	-
14	6
9	3

Mal

+	-	V
1	0	23
9	1	26

11^{30} - 5PM.

ca 200/ml. About 2 clones / plate.
Agues with 2-3 ~~pla~~ strains not required.
On EMB Lac 6 plates. EMB Mal 3 plates

Clones:

—		EMB Mal
	1^1 V	1 V
1	1^4	1 V
		1 +
	1^{10}	1 V (+, - also!)
	no segregants	1 mixed V, +.

No useful data but method is substantiated.

8/5/51. H226 A) .01 ml 10^{-6} B) .01 ml 3×10^{-7}
D . Lac count; many —. (assay: 14 V, 17 —)

5 usable Lac V clones, ca 10/. 2 possible segregants, but crowded.
Streaks on EMB Mal. (Both Mal +) ✓

8/6/51. As above. Total 25 plates, mostly too crowded Mostly Lac V
E 47 possible useful clones (5-20) ~~a~~ altogether. (5½ h. mic.)
Pick Lac - to EMB Mal · 12 : all Mal +. (2 Mal V)

8/7/51 4½ h. mic. ~~Loop~~ standard loop method — Few clones
F should use 2 x. Many Lac - !
(over)

G, H. UV 20 secs. ca 50% surv.

Results inconclusive. Increase in Mal+ in both intact and resp'ed.

Method probably is unsound.

auxotroph diploids. 868

July 30, 1957 FA

A. H226, grown in D(Lac), no treatment. auxotrophy noted in replica platings. Nutrition, Mal, S tested in such platings.

H267, grown D(Lac). UV 30 secs. Grew heavily in D(Lac) +

B. BM

C. TLB$_1$.

Plate on EMB Lac, Replica tests for auxotrophies. B gave numerous auxotroph Lac$^-$; C very few.

	Type		Nutr	Lac	Mal	S	Mal fermd.	S
A.		1	M	V	V [redacted]	[redacted] S		H294
	6	2	TL	V	+ V	S	H245 type	
	6	3	TL	V	+ V	S		

			Nutr						
B	3	1	M	TL	−	−		−	R
	4	2	TL	M	−	−	V	−	R
	1	3	M	TL	+	−		[redacted] V	[redacted]
	5	4	M	TL	V		S	V	S
	1	5	M	TL	+	+ V		V V	[redacted]
	6	6	TL	M	V		S	V	S
	3	7	M	TL	−	−		−	R
	−	8	+	+	+	−		V	S
	4	9	TL	TL	−	−		−	R
	3	10	M	M	−	−		−	R
	5	11	M	M	−V		S	(V)	S
	5	12	M	M	+V		S	(V)(+)	S
	−	13	MTL	MTL ?	+	V		V	V
	1	14	M	TL	+V	−		V	V
	3	15	M	TL	−	−		−	R
	4	16	TL	M	−	−		−	R
	3	17	M	TL	−	−		−	R
	1	18	M	TL	V	−V		V	V
	−	19	+	+	−	−		−	R
	3	20	M	TL	−	−		−	R
	−	21	MTL	MTL ?	+		S	(+)	S
	3	22	M	TL	+	−		−	R
	3	23	M	TL	+	−		−	R
C	2	1	TL		V	−		V	V
	2	2	TL		+	−		V	V
	2	3	TL		+	−		V	V

		Mal	S	
A1	M	V?	S	= H294
A2	TL	V	S	
B1	M	—	R	H291
B2	TL	—	V	
B3	M	V	V	—
B4	M	V	S	(H294type)
C1	TL	V	V	

Cross Attempts with E coli B
Phage T3

August 3, 1951.

Mix 1 drop culture or phage T3 (10^{-3} lysate) to 5 ml Penassay
Incubate overnight.

A 8/3

1	Y44		
2	Y51		
3	Y44 + Y51		
4	Y44 + T3	clear	
5	Y51 "	"	
6	Y44 + Y51 + "	"	

→ #1, 2, 3, 6 plated. all grew without plaques on EMB.
No protoplasts after 7 hours.

Sediment turbid cultures. Plate on D(o)

Repeat A, with heavier bacterial inoculum (ca. 4/ml)

1-3 turbid. 4-6 clear. Streak out 4-6. Also W1664/3 passages

B 8/4

4-6 remain clear!
No true 1664/3 formed as survivors (phage tests)

W1663/3 obtained from platings on EMB. = W1679.
Purify by 3 streakings on EMB and retube —.

C 8/12.

1	1679		Protoplasts? 1
2	" + T3	±	1
3	1679 + 1665		1
4	1665		2
5	1665 + T3	Clear.	
6	1679 + 1665 + T3.	± turbidity.	3

Sediment. Also inoculate 2 and 6 into Penassay.

a) The parents are not completely stable
b) No detectable effect. The crosses had numerous ● (L?) forms.

See Notes by E. Cohn

August 29 (ff.) 1957.

a) Diauxotrophs from PF9 (meth-) and PF12 (leuc SR).

UV 40sec., resuspended cultures in water then with Lederberg broth Wash ↑ once 1:100 in D(c) + meth + leuc + 1000u/ml penicillin, ↑ aerate overnight. Plate out MB lac after 10-12h., replica to D(ML).

PF 9 ca 15%. auxotrophic.

PF 12 ca 5%. Repick to EMB for Recheck.

On Recheck:

PF9. 7/8 and 37/40 = 44/48 OK as auxotroph

PF12 14/20 OK.

See 870a. for Random series.

b) Plate irradiated suspensions from (a) at ↑. PF9, PF12 and PF9+12 (uv, grown together). The last showed high counts of prototrophs (ca 5000/ml) but no such counts (50 1000?/ml) were seen in the separate cultures also.

Physlia Plate Tests

8/09

		Group	HC	Vits	A1	A2	A3	A4	A5	YNA	EMB
PF13 (1)	1	A2				+					
(2)	2	3					+				
(3)	3										
(4)	4	4						++			
	5	2				+					
	6	3					+				
	7	2				+					
	8	3					+				
	9	2				+					
	10	2				+					
	11	2				+					
	12	3					+				
	13	2				+					
PF9 (1)	1	2				+					
(2)	2	3					+				
	3	2				+					
	4	2				+					
	5	2				+					
	6	2	x			+					
	7	2				+					
	8	3					+				
	9	3				± ?	+				
	10	2				+					
	11	3	all but 53	None x			+				
	12	3					+				
	13	2				+					
	21	3					+				
	22	2				+					
	23	3					+				
	24	2				+					
	25	3					+				
(3)	26	4				+		++			
	27	2				+					
	28	2				+					
(4)	29	1			++						
	30	2				+					
	31	2				+					
	32	2				+					
	33	2				+					
	41	[redacted]	[redacted]	[redacted]	[redacted]	+		[redacted]	±	++	++
	42	2				+					
	43	2				+					
(5)	44	1			++	+					+
	45	2				+					
	46	2				+ +	+ −				
	47	3				+ −	+				
	48	2				+					
	49	2				+					
	50	2				+					
(6)	53	YNA				+		See over		±	
	[redacted] AH ERRATIC.										

all A3 – Rechecked by replica:
all were Trypt. –
Discard these, and all A2 – (presumably added. Isol r Val or IV)

and PF 26.

Keep other PF numbers and preserve in dry tubes.

September 4, 1951.

	PF									
PF12:	19	L+: A2	Iso	Val	I.V.			0		Iso-Val
add leuc	20	A3	A3	φal	tyr	tryp.	φal+tyr	0		Tryp
	21		HCV	Yeast	RNA	NZ.		0		??
	22	A4	A4	hist	thre	glut	[illegible]	[illegible]	0	Hist
PF 9:	23	A2 M+: A2	L.	IL	V.L	I+V.	I+V+L	0		IsoVal
add meth.	24	A3	A3	φal	tyr	tryp	φal+tyr	0		Tryp
	25	A4	A4	hist	thre	glut	[illegible]	[illegible]	0	Hist
	26		A1	Lys	Arg	Cyst	0			Cyst
	27		A1	Lys	Arg	Cyst	0			Cyst
	28	YNA. M+ YNA.	Pur	Pyr	Guan	Aden	Xanth	Hypx	RNA (Guanine)	0

For crossing, try Meth Cyst × Leuc Isol. PF 26 × PF19.

These cultures preserved by drying by Hershey's method. Also try non-developmental experiments. Add suspension directly to granular or powdered SiO_2 gel, previously sterilized and standing in tubes.* Seal off in air unless otherwise indicated on tubes.

9/4-5. Attempt crosses of PF 26× 17. a) Plate zygote [illegible] cultures b) Grow together in P. assay; c) UV of cells. or washed cells, inoculate P. in assay sep. and together.

(Record of date by registered letter.)

Crossing Attempts with Pseudomonas fluorescens 870c

Sept 4, 1951. ff.

see 870b.

PF 26 × 19
Inc plates 3-4 days.

a. Grown separately.

4/4	1	19	0 0
	2	26	0
	3	26 + 19	5 v. small (cont ??) PF ??

b. Grown together

9/5	1	19	0
	2	26	10 !
	3	(26 + 19)	2 .
	4	(26) + (19)	1

4 PF
PF.

c. UV 60s; grown together

	1	19	0
	2	26	0
V. heavy	3	(26 + 19)	1, 2? (pinpoints, microcolonies)
	4	(26) + (19).	2' Not PF. Papillate clones! Not PF

Hold in refrigerator for later study.

1/16/51. Streaked out D(0):

1 } "a 3"
2 }
3 } b 2
4 }
5 }
6 }
7 } 63
8 }

all S's

Pseudomonas ??? doubtful.

9 } b 1
10 }

D. 1. PF 9 = M- 6 5 petrotypes.

2. PF 19 UV L-IV-S^R 0

3. PF 19 UV + PF 9. 3 large + many small → >100 petrotypes.

4 = 1+2 4 + (1-2) 9 "

Replica to EMS tube sm: None SR

This result is rather indecisive. ① Repeat with tests for S^R ... see 769

② Survey other mutants for stability. Test PF 2, 3, 4, 7, 8

Use PF 12 = L-S^R × PF 28 = M-Su-

P. fluorescens

9/21/51. Stability tests on auxotrophs.

Grow on NA. 48 hrs. Harvest, wash and suspend in 3± ml. Plate 1 ml samples on minimal agar. (probably ca 10^9 cells)

	Prototrophs
PF 2	9
3	0 , same background (Marin ?)
4	0 " " "
6	> 100
7	> 500
8	10
12	21
28	ca 30 (for Guan + Meth)

Monoauxotrophs of choice are [redacted], PF3 , (4)

Try × PF 28

10/2. PF28 (uv) × PF12. SM

PF12	9	T^R 3^S
PF28uv	0	—
from together	3	3R
" separately.	2	3R

No evidence of recombination.

Replica to EMS ± Sm.

Is PF12 homogeneous?

10/6: Yes!!

Experiment should be repeated

10/3 Stock culture streaked out on EMB Lac. Colonies replica plated to EMB Lac Sm. All of ca 50-100 were SR.

The PF12 S^S may have been contaminants

10/F. Nitrate Purnassay cultures. (PF-12 from colony on Sm.)

Expt. completed ca 10/20. Just as above.

PF12	ca 50	all SR	By replicate
" 28uv	0		
" 12+28uv	ca 50.	all SR	"

No evidence of recombination!

Pseudomonas fluorescens

870s.

Summary of Crossing Expts.

10/22/51.

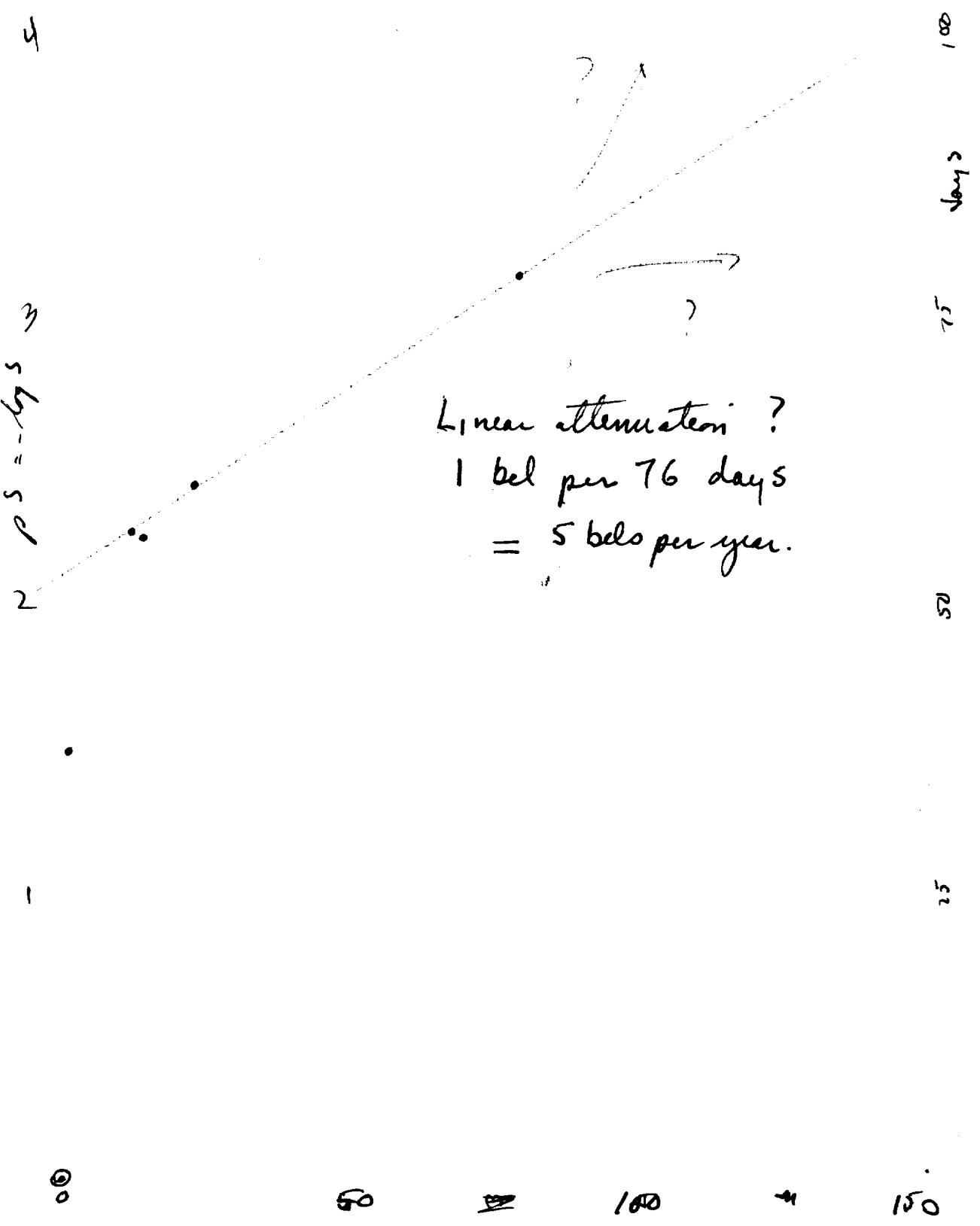
Diauxotroph combinations: L-IV- M-Cys PF 19 x 26

PF 26 gave a few prototrophs on dense platings.

Mono-diauxotroph combination (SR mutant):

PF 19

Try PF 19 x 28.


4
3
2
1
0
50
100
150
100
75
50
25
days
Linear attenuation ?
1 bel per 76 days
= 5 bels per year.

Solid adsorbent for preserving cultures

September 4, 1951.

a) See typewritten memorandum

1/5. b) Dry K-12 by various methods to compare viability. (From heavy suspensions re-equal to peptone 2% from NA slants).

			9/18/51 contents/10ml broth
1. $CaCl_2$ etc. (Hershey, Brown)			2.5×10^7
2. In contact with granular silica,		air	2.5×10^5
3	" "	vac.	
4	" powdered	air	
5	" "	vac.	

1/4 c) 6. PF 21

1. gran SiO_2 air
2. " SiO_2 vac.

9/5. Open 1, 2, suspend in 10ml Pennassay and titrate. (10^{-7})

c1: 2 c2: 17

872
B

September 30, 1951.

Resume: Survival.

		Days		pS	Count: 1.1/10 .1/10 .1/10 } .1 ml = 1x 10^{-6} ml from 10 ml
9/6/51.	Initial	0	3.4×10^9/ml [10 ml]	0 (9.53)	340
9/7		1	11×10^7	1.49	11 1; 187/100
9/14		12	1.9×10^7	2.25	
9/15		14	$.2 \times 10^7$	2.23	20/10
9/29		23	1.3×10^7	2.42	13/10; 1/1; 80/100.
11/24	~~[illegible]~~	79	2.4×10^5	~~[illegible]~~ 3.14	1/10 17/100 245/1000

September 6, 1951.

1 K12, 10 ml Penassay, aerated overnight. A5 II A1 I

A. Broth, direct.

B. ", sedimented, resuspend in 1 ml (1:10)

.05 ml per tube.

1.	Fran silica, Air		~~HH~~II
2	" "	Vac	II
3.	Pdr Alumina	Air	
9/6/51. 4.	" "	Vac	
5	Hershey $CaCl_2$ - Vac		I

Assay B. (.05/10; etc.). Count 299

1.1/10 376

.1/10 675

.1/10 .1

(= 10^{-6} ml sample from 10 ml.) Original titer 6.7×10^{9} cells/ml.

Each tube received 3.4×10^{9} (3.4×10^{8}) conc. 10:1 direct broth.

Hold in refrigerator

9/7/51. 872 B1 assay sample tubes. Empty tubes into 10 ml Penassay, including washings from walls. Dilution schedule as above: (.05/10); 1.1/10; .1/10, .1/10. Plate .1

		Count	10×	100×	Assay/ml (in 10 ml)		Survival (pS)
A	1		2	15	2×10^{5}		1.3
	2		15	121	1.5×10^{6}		.3 !
B	1		11	161	1.4×10^{7}		1.3
	2		10		10^{7}		1.3
	3		0		0		—
	4		0		0	—	—
	5		14	188	1.4×10^{7}		1.3

340.

Ca 95% "Process loss" and attenuation in 24 hours.

9/14/51.	~~B2~~. B1	1:2)	11	187	1.9×10^{7}	2.2
9/15/51	B1 B5		20 / 0	0 !	~~[illegible]~~ 7	~~2.4~~

September 15, 1951.

Harvest from Permessay flasks. Concentrate in [illegible] 20°.
Ca. 30/1.5 = 20:1 Use .05ml per sample ē 1ml [illegible]

a) Titrate initial samples (1.1/10; .1/10; .1/10; .1/plate)

Silica tubes previously baked, store in [illegible] over Drierite.

		Count	Assay/tube
Initial assay. A.	58-161	34	$.34 \times 10^9$
	W-1177	148	1.5×10^9

Until 10/11/51, Silica used was Davison 05-08-09-216.

New lot received 10/11/51 (Grade); 40-08-09.226

10/11/51.

B. ~~[illegible]~~ H295. Harvest, 10:1, .05 ml/tube. Assay $\frac{100 \times 139 \times 10^{-8}/2}{ml}$ $= 3.5 \times 10^9$ particles

	Count.	Surv.
1. Old Silica	33	
3. New Silica	< 10	
2. Silica Grade 923 Mesh 100-200.	< 10.	
4. Activated Alumina	1-2	

10/12/51 ~~Plate~~ Add tube to 10ml. Plate 10^{-5} ml

Very low survivals. Should be repeated.

Department of Genetics
University of Wisconsin
Madison 6, Wisconsin

January 1, 1952

Preservation of Bacterial Cultures on Silica Gel

This circular was written in response to a number of inquiries. Judging from these, present methods for preservation of bacterial cultures are not entirely satisfactory, and it would be a real contribution to laboratory technique to work out a better one. Unfortunately, I can only suggest a principle that seems very plausible, but that has not yet been empirically justified.

The working principles are (1) that suitably dried bacteria should survive just as well in a sealed tube under air as in vacuo, and (2) that if this is correct, chemically inert desiccants such as anhydrous silica gel could greatly facilitate the practice. The following arrangement has been tried: small vials or tubes are filled nearly full with silica gel (Davison Company, Baltimore; Grade 40, 6-16 mesh). About 1 to 1.5 gms. of silica fits well into the tubes used. The tubes are plugged, then baked in a sterilizing oven at 160-180 C., 2 hours, to dry and sterilize the tubes. These are stored in a desiccator. The bacteria to be preserved are suspended in 2% peptone. About .05 ml. is pipetted directly to the silica, and the end of the tube sealed off. The tubes were then stored in a refrigerator. The water disappears very quickly. To regenerate the cultures, the tubes were broken, and the silica poured into broth. Considerable gas is liberated. After about an hour to allow redispersion, viable counts were made on the broth. The 24-hour survival, in apparently dried condition, was quite high (about 10%), but this was more encouraging than long-term experiments. After four - five months, the survival has been low, of the order of 10^{-5}, and some tubes are inviable. In its present form, the method is not a success, and cannot be recommended for long-term preservation and storage. I think that it could be greatly improved, without complication, by experiments leading to a better suspending fluid, and possibly by drying the cells on a layer of glass bead over the silica. What is most needed is an improved theoretical understanding of the biology of suspended animation in successfully dried cultures.

Despite its shortcomings, the silica gel tubes provide an ideal method for mailing cultures. They are not affected by undue cold in the way agar slants are, and probably ought to be more resistant to high temperatures as well. The mechanical strength of small sealed tubes allows them to be sent with simple padding in an ordinary envelope, and the absence of any liquid minimizes possible hazards from breakage and leakage.

I hope that other workers with suitable facilities to study preservation problems, or with a potential interest in the biophysics of suspended animation may be able to make some use of this suggestion.

Joshua Lederberg

September 25, 1951.

A. K-12 overnight, aerated. Dilute 1:10 (est. 2×10^8/ml)

A 2 .02 ml Assay original K-12: 5.7×10^9

A 5 .05 ml

B2 1:1000 (est 2×10^6/ml). .02 ml

Assay. B2. ① Ca 2 gm. Silica ② Ca .5 gm Silica

4/29/51. A2 ③ " ④ "

2 gm samples may have been exposed to heat glove to cell during sealing.

		Survival	pS
1	6	6/114	
2	16	16/114	
3	8.75	8.75/114	1.115
4	> #3 ca 10%		1 ±

A2 init 1.14×10^6/tube. 11,400 per plate = pS 0.

B2 init. 114.